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FATTY ACID ESTERS FROM THE ROOTS OF *GLYCYRRHIZA GLABRA* L.

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ABSTRACT

Glycyrrhiza glabra L. (Family: Fabaceae) is a herbaceous perennial legume native to northern Africa, southern Europe and western Asia. Its roots are mainly used to treat arthritis, asthma, colic, bronchial catarrh, bruises, burns, colds, coughs, diabetes, diarrhoea, eczema, epilepsy, fever, flatulence, gastric and mouth ulcers, haematemesis, hair fall, haemorrhagic diseases, hepatitis, herpes, hyperdipsia, indigestion, influenza, jaundice, kidney stones, leucorrhoea, lung ailment, malaria, menstrual disorders, oedema, ophthalmia, paralysis, prostate cancer, psoriasis, rheumatism, sexual debility, skin eruptions, sore throat, stomach ulcers, tonsillitis, tuberculosis, vomiting and wounds. Our study was planned to isolate chemical constituents from the roots of *G. glabra* and to characterized their structures. Phytochemical investigation of the roots led to the isolation of fatty acid esters identified as *n*-octadecanyl hexanoate (**1**), *n*-heptadecan-16′-one-17′-ol-1′-olyl stearate (**2**), *n*-tetratriacontanyl acetate (geddyl acetate, **3**), *n*-pentadecanyl tetracosanoate (*n*-pentadecanyl lignoceroate, **4**), *n*-dodecanyl *n*-octacosanoate (lauryl montanite, **5**), *n*-octadecyl *n*-docosanoate (stearyl behenate, **6**), *n*-nonatriacontanyl butanoate (*n*-nonatriacontanyl butyrate, **7**) and *n*-butyl *n*-tetracontanoate (**8**). The structures of isolated phytoconstituents were established on the basis of analysis of spectral data.

KEYWORDS: *Glycyrrhiza glabra* L., roots, fatty acid esters, isolation, characterization.

INTRODUCTION

Glycyrrhiza glabra L. (Family: Fabaceae), commonly known as liquorice, mulaithi or yashtimadu, is a herbaceous perennial legume native to northern Africa, southern Europe, and western Asia. It grows up to 1 metre in height, with stoloniferous, internally yellow, sweet roots, odour typical; imparipinnate, alternate, lanceolate leaves, leaflets 9-17; flowers purple to pale blue, papilionaceous, axillary, in a loose inflorescence; fruit is an oblong, glabrous pod; seeds several, brown, reniform. The roots and rhizomes are anti-allergic, antidysenteric, anti-inflammatory, antispasmodic, antisyphilitic, anti-tussive, carminative, cystitis, demulcent, diuretic, emollient, expectorant, fungicide, pectoral, purgative, ulcer protective and tonic; used to treat Addison disease, adrenocorticoid insufficiency, allergic states, anaemia, arthritis, asthma, colic, bronchial catarrh, bruises, burns, colds, coughs, diabetes, diarrhoea, eczema, epilepsy, fever, flatulence, gastric and mouth ulcers, haematemesis, hair fall, haemorrhagic diseases, hepatitis, herpes, hyperdipsia, indigestion, influenza, jaundice, kidney stones, leucorrhoea, lung ailment, malaria, menstrual disorders, oedema, ophthalmia, paralysis, prostate cancer, psoriasis, rheumatism, sexual debility, skin eruptions, sore throat, stomach ulcers, tonsillitis, tuberculosis, vomiting and wounds.[1-5] It is taken as a food and beverage flavouring agent and added for flavour enhancing and moistening agents in the manufacture of blend cigarettes and other tobacco products with a natural sweetness and a distinctive flavour.[1-5]

Liquorice roots contained triterpenoid saponins mostly glycyrrhizin, potassium and calcium salts of 18β-glycyrrhizic acid (it was 50 times sweeter than sugar), triterpenes, e.g., liquiritic acid, glycyrretol, glabrolide, isoglaborlide, 22β-acetoxyl glycyrrhizin, liquorice acid and ergosta-7,22-dien-3β,5α,6β,9α-tetraol, licorice saponin L 3, lipinifolin,[6-8] phenolic contents, flavonoids and chalcones, e.g., liquiritin, liquiritigenin, hamnoliquiritin, neoliquiritin, isoliquiritin, isoliquiritigenin, neoisoliquiritin, licuraside, glabrolide, licoflavonol, 2,2′,4′-trihydroxy-chalcone, 5,8-dihydroxy-flavone-7-O-beta-D-glucuronide, glychionide A, 5-hydroxy-8-methoxylflavone-7-O-beta-D-glucuronide and glychionide B, isoflavones: glabridin, galbrene, glabrone, shinpterocarpin, licoisoflavones A and B, formononetin, glyzarin, kumatakenin, hispaglabridin A, hispaglabridin

B, 4′-O-methylglabridin, 3′-hydroxy-4′-O-methyl glabridin, glabroisoflavanone A and B, 2-O-β-D-xylopyranoside, (3- methoxy phenyl) 4'-methoxy, 3',5' dihydroxybenzofuran, 5,2',4'-trihydroxy, 8,3'-dimethoxy 4',5' - dihydroxyisopropyl dihydrofuran flavanol, isolicoflavanol, 7-O-β-D-xylopyranosyl (1→4) β-D-glucopyranosyl 6-O-isobutyl isoflavone, licochalcone B, hispaglabridin B, 4-O-methyl glabridine, shinflavanone, licoflavonol, licoricidin, licoisoflavanone, isoglycycoumarin, semilicoisoflavone B, and 3-methoxy-9-hydroxy-pterocarpan,[7, 9-16] oleanane-type triterpenoid saponins (glabasaponin A-G),[17] essential oil consisted mainly of isoniazid, diethyltoluamide, benzoic acid, benzene, linalool, prasterone, warfarin, iodoquinol, 4-(2-aminopropyl) phenol, hexanoic acid, hexadecanoic acid, hexanol and octanoic acid, estragole (methyl chavicol), anethole, eugenol, indole, γ-nonalactone and cumic alcohol,[18,19] (E)-2-heptenal, 5-methyl- furfural, heptadienol, (E)-2-octen-1-al, o-guaiacol, 2-phenylethanol, (Z)-pinene hydrate, lavandulol, terpinen-4-ol, (E)-linalool oxide, p-cymen-8-ol, α-terpineol, methyl chavicol, (4E)-decenal, decanal, nonadienal, cumin aldehyde, carvone, piperitone, cinnamaldehyde, anethole, decadienal, thymol, carvacrol, decadienal, p-vinyl guaiacol, eugenol, methyl eugenol, β-caryophyllene, β-dihydro-ionone, himachalene epoxide, spathulenol, (1α, 10α)-epoxy-amorph- 4-ene, β-caryophyllene oxide and humulene epoxide II.[20] The plant contained many amino acids including aspartic, glutamic, threonine, serine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, tyrosine and lysine; organic acids including acetic, fumaric, butyric, propanoic, malic, citic and tartaric acids.[21] The leaves possessed dihydrostilbene derivatives[22] and flavanones.[23]

The presence of herbal chemical constituents vary due to many factors such as soil, geographic regions, seasonal changes, plant species and application of fertilizers. Keeping in views the various therapeutic values, variation aspects of chemical constituents of the plants and development of ecofriendly, biodegradable and safer herbal preparations the roots of *Glycyrrhiza glabra* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[24-26]

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were scanned on a Bruker DRX (400 MHz) instrument using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/*z values of the more intense peaks are mentioned and the figures in bracket attached to each *m/*z values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). Purity of the compounds was checked by TLC over silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant material

The roots of *Glycyrrhiza glabra* were procured from the local market of Khari Baoli, Delhi. The drug material was identified by Prof. M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen of the drug is preserved in the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

Extraction and isolation

The pulverized root powder (1.0 kg) was extracted exhaustively in a Soxhlet apparatus with methanol. The combined extract of the drug was dried under reduced pressure to secure a viscous dark brown residue (117 g). A small portion of the extract was analyzed chemically to determine the presence of different types of chemical constituents. The dried residue (100 g) was dissolved in a minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain a slurry. The slurry was air-dried and chromatographed over a silica gel column loaded in petroleum ether (b. p. 60 – 80 °C). The column was eluted with petroleum ether, petroleum ether – chloroform (9:1, 3:1, 1:1, 1:3, *v/v*) and chloroform. Various fractions were collected and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

n-Octadecanyl hexanoate (1)

Elution of the column with petroleum ether furnished a colourless mass of **1**, 201 mg, R_f 0.91 (*n*-butanol – glacial acetic acid-water, 4:1:1); IR υ_{max} (KBr): 2966, 2842, 1725, 1637, 1455, 1363, 1260, 1026, 975, 800 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.17 (2H, t, J = 6.8 Hz, H_2-1′), 2.50 (2H, m, H_2-2), 1.84 (2H, m, H_2-3), 1.35 (4H, m, H_2-2′, H_2-3′), 1.28 (32H, brs, 16 x CH_2), 0.86 (3H, t, J = 6.9 Hz, Me-18′), 0.81 (3H, t, J = 6.6 Hz, Me-6); ^{13}C NMR ($CDCl_3$): δ 173.01 (C-1), 60.89 (C-1′), 34.92 (C-2), 30.54 (C-3), 29.89 (17 x CH_2), 21.08 (C-2′), 19.01 (C-6), 14.21 (C-18′); +ve ESI MS *m/z* (rel. int.): 368 $[M]^{+}$ ($C_{24}H_{48}O_2$) (39.5), 269 (100).

***n*-Heptadecan-16′-one-17′-ol-1′-olyl stearate (2)**
Elution of the column with petroleum ether – chloroform (9:1) gave a colourless sticky mass of **2**, 118 mg, R_f 0.83 (*n*-butanol – glacial acetic acid-water, 4:1:1, v/v); IR υ_{max} (KBr): 3215, 2964, 2831, 1722, 1690, 1637, 1425, 1261, 1050, 973, 796 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.05 (2H, m, H_2-1′), 3.37 (2H, s, H_2-17′), 2.51 (2H, m, H_2-2), 2.28 (2H, m, H_2-15′), 1.80 (2H, m, H_2-3), 1.36 (4H, m, H_2-4, H_2-14′), 1.25 (52H, brs, 26 x CH_2), 0.83 (3H, t, J = 6.9 Hz, H_3-18); ^{13}C NMR ($CDCl_3$): δ 189 (C-16′), 168.35 (C-1), 61.08 (C-1′, C-17′), 34.94 (C-2), 29.98 (C-3), 28.83 (27 x CH_2), 22.30 (C-17), 14.67 (C-18); +ve ESI MS *m/z* (rel. int.): 552 $[M]^+$ ($C_{35}H_{68}O_4$) (6.8), 285 (20.3), 267 (11.2).

Geddyl acetate (3)
Elution of the column with petroleum ether – chloroform (1:1) afforded a colourless mass of **3**, 192 mg, R_f 0.76 (*n*-butanol – glacial acetic acid-water, 4:1:1); IR υ_{max} (KBr): 2928, 2837, 1721, 1643, 1448, 1305, 1150, 1097, 974, 797, 727 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.12 (2H, t, J = 6.6 Hz, H_2-1), 2.15 (3H, brs, $COCH_3$), 1.55 (2H, m, H_2-2), 1.35 (30H, brs, 15 x CH_2), 1.25 (32H, brs, 16 x CH_2), 0.83 (3H, t, J = 7.2 Hz, Me-34); ^{13}C NMR ($CDCl_3$): δ 173.06 (C-1′), 60.91 (C-1), 34.89 (C-2), 30.52 (C-3), 29.91 (30 x CH_2), 21.59 (C-33), 18.98 (C-2′), 14.17 (C-34); +ve ESI MS *m/z* (rel. int.): 536 $[M]^+$ ($C_{36}H_{72}O_2$) (100), 493 (98.7).

***n*-Pentadecanyl lignoceroate (4)**
Further elution of the column with petroleum ether – chloroform (1 : 1) produced a colourless mass of **4**, yield 203 mg, recrystallized from chloroform-methanol (1:1), m. p. 66 - 67 °C; R_f 0.58 (*n*-butanol-gl. acetic acid – water, 4:1:1); IR υ_{max} (KBr) : 2927, 2838, 1725, 1639, 1435, 1260, 1135, 1118, 971, 798 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.08 (2H, t, J = 6.9 Hz, H_2 -1′), 2.25 (2H, t, J = 7.2 Hz, H_2 -2), 2.05 (2H, m, H_2-3), 1.55 (2H, m, H_2-2′), 1.34 (4H, m, H_2-4, H_2-5), 1.29 (40H, brs, 20 x CH_2), 0.87 (3H, t, J = 7.2 Hz, Me-24), 0.82 (3H, t, J = 7.0 Hz, Me-15′); ^{13}C NMR ($CDCl_3$): δ 172.79 (C-1), 60.87 (C-1′), 35.01 (C-2), 21.56 (21 x CH_2), 27.69 (C-21), 25.24 (C-22), 22.62 (C-23), 19.02 (Me - 24), 14.19 (Me -15′); ESI MS *m/z* (rel.int.): 578 $[M]^+$ ($C_{39}H_{78}O_2$) (9.3), 367 (13.2), 351 (4.8).

Lauryl montanate (5)
Elution of the column with petroleum ether – chloroform (1 : 3) gave a colourless mass of **5**, yield 186 mg, recrystallized from chloroform-methanol (1:1), m. p. 71 - 72 °C; R_f 0.60 (n-butanol-gl. acetic acid – water, 4:1:1); IR υ_{max} (KBr) : 2931, 2842, 1727, 1637, 1432, 1263, 1097, 974, 796 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.11 (2H, t, J = 6.6 Hz, H_2 -1′), 2.21 (2H, t, J = 7.5 Hz, H_2 -2), 1.98 (2H, m, H_2-3), 1.41 (4H, m, H_2-2′, H_2-4), 1.36 (6H, m, H_2-5, H_2-6, H_2-7), 1.27 (58H, brs, 29 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-28), 0.83 (3H, t, J = 6.9 Hz, Me-12′); ^{13}C NMR ($CDCl_3$): δ 172.76 (C-1), 60.89 (C-1′), 55.41 (C-2), 35.01 (C-3), 31.01 (31 x CH_2), 27.73 (C-25), 25.27 (C-26), 21.57 (C-27), 19.01 (C-12′), 14.17 (C-28); ESI MS *m/z* (rel.int.): 592 $[M]^+$ ($C_{40}H_{80}O_2$) (18.3), 407 (2.8), 185 (2.1).

Stearyl behenate (6)
Further elution of the column with petroleum ether – chloroform (1 : 3) yielded a colourless mass of **6**, yield 308 mg, recrystallized from chloroform-methanol (1:1), m. p. 66 - 67 °C; R_f 0.88 (n-butanol-gl. acetic acid – water, 4:1:1); IR υ_{max} (KBr) : 2972, 2839, 1725, 1635, 1425, 1336, 1259, 1095, 970, 798 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.17 (2H, t, J = 6.8 Hz, H_2 -1′), 2.27 (2H, t, J = 7.3 Hz, H_2 -2), 1.88 (2H, m, H_2-3), 1.54 (4H, m, H_2-2′, H_2-4), 1.34 (10H, brs, 5 x CH_2), 1.24 (54H, brs, 27 x CH_2), 0.83 (3H, t, J = 7.2 Hz, Me-22), 0.83 (3H, t, J = 7.0 Hz, Me-18′); ^{13}C NMR ($CDCl_3$): δ 172.81 (C-1), 61.04 (C-1′), 55.37 (C-2), 34.54 (C-3), 30.39 (31 x CH_2), 27.69 (C-19), 25.18 (C-20), 21.45 (C-21), 18.85 (C-22), 14.01 (C-16′); ESI MS *m/z* (rel.int.): 592 $[M]^+$ ($C_{40}H_{80}O_2$) (8.6), 323 (5.6), 269 (2.3).

***n*-Nonatriacontanyl butyrate** (**7**)
Elution of the column with chloroform afforded a colourless mass of **7**, yield 273 mg, recrystallized from acetone-methanol (1:1), m. p. 92 - 94 °C; R_f 0.91 (*n*-butanol-glacial acetic acid – water, 4:1:1); IR υ_{max} (KBr) : 2976, 2841, 1723, 1639, 1435, 1261, 1076, 972, 797 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.06 (2H, t, J = 6.3 Hz, H_2 -1′), 2.24 (2H, t, J = 7.2 Hz, H_2 -2), 1.87 (2H, m, H_2-3), 1.57 (4H, m, H_2-2′, H_2-4), 1.38 (4H, m, H_2-5, H_2-6), 1.24 (72H, brs, 36 x CH_2), 0.87 (3H, t, J = 7.2 Hz, Me-4), 0.82 (3H, t, J = 7.0 Hz, Me-39′); ^{13}C NMR ($CDCl_3$): δ 172.74 (C-1), 60.87 (C-1′), 35.01 (C-2), 34.54 (C-3), 30.39 (34 x CH_2), 27.58 (C-36′), 25.28 (C-37′), 22.71 (C-38′), 19.02 (C-4), 14.17 (C-39′); ESI MS *m/z* (rel.int.): 634 $[M]^+$ ($C_{43}H_{86}O_2$) (2.1), 563 (9.2).

***n*-Butyl *n*-tetracontanoate (8**)
Further elution of the column with chloroform furnished a colourless mass of **8**, yield 182 mg, recrystallized from acetone-methanol (1:1), m. p. 112-114 °C; R_f 0.66 (*n*-butanol- glacial acetic acid – water, 4:1:1); IR υ_{max} (KBr) : 2951, 2841, 1721, 1637, 1435, 1251, 1192, 1028, 977, 798 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.37 (2H, t, J = 6.8 Hz, H_2 -1′), 2.50 (2H, t, J = 7.4 Hz, H_2 -2), 1.88 (2H, m, H_2-3), 1.54 (4H, m, H_2-2′, H_2-4), 1.37 (4H, m, H_2-5, H_2-6), 1.24 (68H, brs, 34 x CH_2), 0.86 (3H, t, J = 6.5 Hz, Me-4′), 0.82 (3H, t, J = 7.0 Hz, Me-40); ^{13}C NMR ($CDCl_3$): δ 173.19 (C-1), 60.93 (C-1′), 34.82 (C-2), 30.79 (36 x CH_2), 27.61 (C-37′), 25.26 (C-38′), 22.69 (C-39′), 18.95 (C-4′), 14.10 (C-40); ESI MS *m/z* (rel.int.): 648 $[M]^+$ ($C_{44}H_{88}O_2$) (16.2), 575 (24.8).

RESULTS AND DISCUSSION

Compounds **1, 3** and **6** were a known fatty acid ester characterized as *n*-octadecanyl hexanoate[27,28], *n*-tetratriacontanyl acetate[29] and *n*-octadecyl *n*-docosanoate.[30], respectively (Fig. 1).

Compound **2** showed IR absorption bands for a hydroxyl function (3215 cm^{-1}), ester group (1722 cm^{-1}), carbonyl

group (1690 cm^{-1}) and long aliphatic chain (796 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/*z 552 corresponding to a molecular formula of a fatty acid ester, $C_{35}H_{68}O_4$. The ion peaks generating at *m/*z 265 [C_1 – O fission, $CH_3(CH_2)_{16}$-$CO]^+$ and 285 [M – 267, OCH_2-$(CH_2)_{14}CO$-$CH_2OH]^+$ suggested the esterification of stearic acid with heptadeca-1′,17′-diol-16′-one. The ^{1}H NMR spectrum of **2** exhibited a two-proton multiplet at δ 4.05 assigned to oxymethylene H_2 -1′ protons. A two-proton singlet was ascribed to hydroxymethylene protons. Two two-proton multiplets at δ 2.51 and 2.28 were attributed to methylene H_2-2 adjacent to the ester carbon and H_2-15′ nearby to the carbonyl protons, respectively. The other methylene protons appeared as multiplets at δ 1.80 (2H) and 1.36 (4H) and as a singlet at 1.25 (52H). A three-proton triplet at δ 0.83 (J = 6.9 Hz) was accounted to terminal C-18 primary methyl protons. The ^{13}C NMR spectrum of **2** showed signals for the ester carbon at δ 168.35 (C-1), oxymethylene and hydroxymethylene carbons at δ 61.08 (C-1′ and C-17′), methylene carbons between δ 34.94 - 22.30 and methyl carbon at δ 14.67 (C-18). On the basis of foregoing spectral data analysis, the structure of **2** has been established as *n*-heptadecan-16′-one-17′-ol-1′-olyl stearate, a new fatty acid ester (Fig. 1).

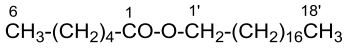
Compound **4**, named *n*-pentadecanyl lignoceroate, showed IR absorption bands for an ester group (1725 cm^{-1}) and long aliphatic chain (798 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 578 consistent with the molecular formula of a fatty acid ester, $C_{39}H_{78}O_2$. The generation of the ion peaks at *m/*z 351 [C_1 - O fission, $CH_3(CH_2)_{22}$-$CO]^+$ and 367 [$C_{1'}$ - O fission, $CH_3(CH_2)_{22}$-$COO]^+$ indicated that a C-24 lignoceric acid was esterified with pentadecanol. The ^{1}H NMR spectrum of **4** displayed two triplets at δ 4.08 (J = 6.9 Hz) and 2.25 (J = 7.2 Hz) integrating for two protons each assigned to oxymethylene H_2-1′ and methylene H_2 -2 protons adjacent to the ester function, respectively. The remaining methylene protons appeared as multiplets at δ 2.05 (2H), 1.55 (2H) and 1.34 (4H) and as a broad singlet at δ 1.29 (40H). Two three-proton triplets at δ 0.87 (J = 7.2 Hz) and 0.82 (J = 7.0 Hz) were due to correspondingly C-24 and C-15′ primary methyl protons. The ^{13}C NMR spectrum of **4** showed signals for ester carbon at δ 172.79 (C-1), oxymethylene carbon at δ 60.87 (C-1′), other methylene carbons between δ 35.01 - 22.62 and methyl carbons at δ 19.02 (C-24) and 14.19 (C-15′). The absence of any ^{1}H NMR signal beyond δ 4.08 and carbon signal between δ 172.79 - 60.87 supported the saturated nature of the molecule. On the basis of above discussion and literature values, the structure of **4** has been elucidated as *n*-pentadecanyl tetracosanoate (Fig. 1).

Compound **5**, designated as lauryl montanite, displayed distinctive IR absorption bands for ester function (1727 cm^{-1}) and long aliphatic chain (796 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/*z 592 corresponding to a molecular formula of a fatty acid ester, $C_{40}H_{80}O_2$. The formation of the ion peaks at *m/*z 407 [C_1 - O fission, $CH_3(CH_2)_{26}$-$CO]^+$ and 185 [M – 407, OCH_2-$(CH_2)_{10}$-$CH_3]^+$ indicated that a C-28 lauric acid was esterified with the montanic acid (octacosanoic acid). The ^{1}H NMR spectrum of **5** exhibited two two-proton triplets at δ 4.11 (J = 6.6 Hz) and 2.21 (J = 7.5 Hz) assigned to oxymethylene H_2-1′ and methylene H_2 -2 protons nearby to the ester function, respectively. The remaining methylene protons appeared as multiplets at δ 1.98 (2H), 1.41 (4H) and 1.36 (6H) and as a broad singlet at δ 1.27 (58H). Two three-proton triplets at δ 0.86 (J = 7.2 Hz) and 0.83 (J = 6.8 Hz) were due to C-28 and C-12′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **5** showed signals for ester carbon at δ 172.76 (C-1), oxymethylene carbon at δ 60.89 (C-1′), other methylene carbons between δ 55.41 - 21.57 and methyl carbons at δ 19.01 (C-12′) and 14.17 (C-28). The absence of any ^{1}H NMR signal beyond δ 4.11 and carbon signal between δ 172.76 - 60.89 supported the saturated nature of the molecule. On the basis of these evidences, the compound **5** was structurally elucidated as *n*-dodecanyl *n*-octacosanoate (Fig. 1).

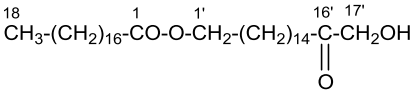
Compound **7**, named *n*-nonatriacontanyl butyrate, showed characteristic IR absorption bands for ester function (1723 cm^{-1}) and long aliphatic chain (797 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 634 consistent with a molecular formula of a fatty acid ester, $C_{43}H_{86}O_2$. The generation of the ion peak at *m/*z 563 [C_1 - O fission, OCH_2-$(CH_2)_{37}$-$CH_3]^+$ indicated that butyric acid was esterified with the nonatriacontanyl alcohol. The ^{1}H NMR spectrum of **7** displayed two two-proton triplets at δ 4.06 (J = 6.3 Hz) and 2.24 (J = 7.2 Hz) assigned to oxymethylene H_2-1′ and methylene H_2 -2 protons adjacent to the ester function, respectively. The other methylene protons appeared as multiplets at δ 1.87 (2H), 1.57 (4H) and 1.38 (4H) and as a broad singlet at δ 1.24 (72H). Two three-proton triplets at δ 0.87 (J = 7.2 Hz) and 0.82 (J = 7.0 Hz) were associated with the C-4 and C-39′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **7** showed signals for the ester carbon at δ 172.74 (C-1), oxymethylene carbon at δ 60.87 (C-1′), other methylene carbons between δ 35.01 – 22.28 and methyl carbons at δ 19.02 (C-4) and 14.17 (C-39′). The absence of any ^{1}H NMR signal beyond δ 4.06 and carbon signal between δ 172.74 - 60.87 indicated the saturated nature of the molecule. On the basis of these evidences, the structure of **7** has been established as *n*-nonatriacontanyl butanoate, a new fatty acid ester (Fig.1).

Compound **8** had characteristic IR absorption bands for an ester function (1721 cm^{-1}) and long aliphatic chain (798 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 648 related to a molecular formula of a higher fatty acid ester, $C_{44}H_{88}O_2$. The generation of a prominent ion fragment at *m/*z 575 [C_1 - O fission, CH_3-$(CH_2)_{38}$-$CO]^+$ suggested that butyl alcohol was esterified with tetracontanoic acid. The ^{1}H NMR spectrum of **8** showed two two-proton triplets at δ 4.37 (J = 6.8 Hz)

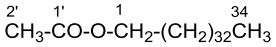
and 2.50 (J = 7.4 Hz) assigned to oxymethylene H_2-1′ and methylene H_2 -2 protons adjacent to the ester function, respectively. The other methylene protons resonate as multiplets at δ 1.88 (2H), 1.54 (4H) and 1.37 (4H) and as a broad singlet at δ 1.24 (68H). Two three-proton triplets at δ 0.86 (J = 6.5 Hz) and 0.82 (J = 7.0 Hz) were due to C-4′ and C-40 primary methyl protons, respectively. The ^{13}C NMR spectrum of **8** displayed signals for the ester carbon at δ 173.19 (C-1), oxymethylene carbon at δ 60.93 (C-1′), other methylene carbons between δ 34.82 – 22.69 and methyl carbons at δ 18.95 (C-4′) and 14.10 (C-40). The absence of any ^{1}H NMR signal beyond δ 4.37 and carbon signal between δ 173.19 - 60.93 supported the saturated nature of the molecule. On the basis of these spectral data analysis, the structure of **8** has been characterized as *n*-butyl *n*-tetracontanoate, a new fatty acid ester (Fig.1).



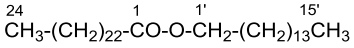
n-Octadecanyl hexanoate **(1)**



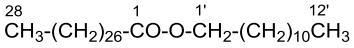
n-Heptadecan-16′-one-17′-olyl stearate **(2)**



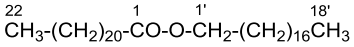
Geddyl acetate **(3)**



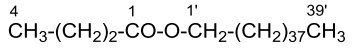
n-Pentadecanyl lignoceroate **(4)**



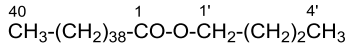
Lauryl montanate **(5)**



Stearyl behenate **(6)**



n-Nonatriacontanyl butyrate **(7)**



n-Butyl *n*-tetracontanoate **(8)**

Fig. 1. Chemical constituents 1 - 8 isolated from the roots of *Glycyrrhiza glabra*.

CONCLUSION

Phytochemical investigation of the roots of *Glycyrrhiza glabra* afforded fatty acid esters identified as *n*-octadecanyl hexanoate (**1**), *n*-heptadecan-16′-one-17′-ol-1′-olyl stearate (**2**), *n*-tetratriacontanyl acetate (geddyl acetate, **3**), *n*-pentadecanyl tetracosanoate (*n*-pentadecanyl lignoceroate, **4**), *n*-dodecanyl *n*-octacosanoate (lauryl montanite, **5**), *n*-octadecyl *n*-docosanoate (stearyl behenate, **6**), *n*-nonatriacontanyl butanoate (*n*-nonatriacontanyl butyrate, **7**) and *n*-butyl *n*-tetracontanoate (**8**). This work has enhanced understanding about the chemical constituents of the plant. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

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